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Biophysical Properties of CX40 Mutants Linked to Atrial Fibrillation
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Gap junctions provide a direct intercellular pathway for cell-to-cell signalling and impulse conduction. The three Cx40 mutations (A96S, M136V and G38D) are associated with atrial fibrillation and retain the ability to form functional channels. The biophysical properties of mutant gap junctions were determined in transiently transfected HeLa and N2A cells. All three mutants exhibited an array of macroscopic coupling ranging from 0.5 to 30 nS and similar voltage dependences (Boltzmann fit: Vj,o = 49, 48, 52 mV, and gj,min=0.20, 0.22, 0.2 for A96S, M136V and G38D, respectively) comparable to wild-type Cx40. However, unitary conductance of G38D channels was 1.76 fold higher than the wild-type Cx40 channel (~220 pS versus ~125 pS). The A96S and M136V mutants exhibited unitary conductances comparable to the wild-type Cx40 (130-140 pS).
The channel permeability was achieved using simultaneous measurement of junctional conductance (gj) and intercellular transfer of a fluorescent probe. All three mutants transferred anionic Lucifer Yellow (LY). The G38D channels exhibited ~9 fold higher anionic LY permeability relative to the ubiquitous cation K^+ (LY/K^+) when compared to the wild-type Cx40 (0.017 versus 0.002), while A96S LY transfer was similar to wild-type (0.003). In contrast, G38D channels were almost impermeable to cationic EthBr (0.001), suggesting that G38D is responsible for altered channel selectivity. Conversely, A96S and M136V channels exhibited enhanced EthBr permeability (0.037 and 0.047 versus 0.013 for wild-type Cx40). Altered conductive and permeability properties of mutant channels suggest an essential role for biochemical and electrical coupling in cardiac tissues. These properties may contribute to regional variability in conduction velocity, cell excitability (ionic-metabolic misbalance), and therefore may be implicated in mechanisms of reentry arrhythmias.
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The Mitochondrial Permeability Transition in Saccharomyces Cerevisiae is Controlled by Hexose Phosphates from the Glycolytic Pathway
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S. cerevisiae is a "Crabtree positive yeast" The "Crabtree effect" is the decrease in oxygen consumption in response to glucose and involves competition between glycolysis and oxidative phosphorylation for ADP and/or Pi. Mitochondrial activity modification by glucose-6-phosphate (G6P), fructose-6-phosphate (F6P) and fructose-1,6-bisphosphate (F1,6BP) appears to be important for the induction of this effect. F1,6BP inhibits the activity of mitochondrial complex III and IV, while is activated by F6P and G6P (Diaz-Ruiz et al. (2008) J Biol Chem, 283, 40, 26948-55). Mitochondrial activity in S. cerevisiae may also be affected by the permeability transition which might be a reversible physiological response mediated by a permeability transition pore (PTP) regulated by cations, ADP, ATP and Pi. In isolated mitochondria from S. cerevisiae we studied the effect of hexoses phosphate on the permeability transition. It was observed that F1,6BP acted as an oxidative phosphorylation coupling agent, while G6P was an uncoupler. It is suggested that the glycolytic intermediaries G6P and F1, 6BP are signals for the communication between glycolysis and oxidative phosphorylation promoting the Crabtree effect.

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ANO1 - a Candidate for Angiotensin-II-Activated Calcium Dependent Chloride Channel in Human Atrial Fibroblasts
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Cardiac fibroblasts are an integral part of the myocardial tissue and contribute to its remodelling. This study characterizes for the first time the calcium-dependent chloride channels (CaCC) in the plasma membrane of primary human atrial cardiac fibroblasts by means of iodide efflux and patch clamp methods. The calcium ionophore A23187 and Angiotensin II (AgII) activate a chloride conductance in cardiac fibroblasts that shares pharmacological similarities with calcium-dependent chloride channels. This chloride conductance is depressed by RNAi-mediated selective of anoctamine 1 (ANO1) but not by Anoctamine 2 (ANO2) which have been revealed as CaCC. The effect of AgII on anion efflux is mediated through AT1 receptors (with an EC50 = 13.8 ± 1.3 nM). Blockade of anion efflux by calphostin C suggests that chloride conductance activation is dependent on PKC. We conclude that ANO1 carries CaCC current in human cardiac fibroblasts and that this is regulated by AgII acting via the AT1 receptor pathway.

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Emerging Electrophysiology of Facultative Bacterial Pathogens
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In contrast to strictly marine microorganisms or obligatory parasites dwelling in a constant environment, facultative pathogens that spend part of their life inside the host and part as free-living forms possess exquisitely robust osmoregulation. Their survival and persistence in highly variable conditions outside of the host defines the pathways and efficacy of transmission. Giant spheroplasts as a system for patch-clamp recording, previously developed for E. coli, already permitted detailed characterization of osmoprotective tension-activated channels in this organism, but homologous channels from other bacteria have never been recorded in their native setting. We have recently developed giant spheroplast preparations for Vibrio cholerae and Pseudomonas aeruginosa. The first patch-clamp survey of both organisms revealed channels with conductances and gating phenotypes similar to MscS and MscL of E. coli, with comparable tension midpoints. Conductive responses to saturating tension are dominated by MscL-like channels in both species. Analysis of genomes pointed to single orthologs of MscL and MscS in V. cholereae, but P. aeruginosa, in addition to MscL, possesses two mscS-like channel genes. Homology models reveal differences from E. coli channels in the distributions of charged and aromatic residues, underlying potentially different interactions with lipids. MS channels in both species are modulated by ampthipathic autoinducers (CAI-1 and PAI-1) from respective bacterial species, which shift their activation curves to the right on the tension scale. This apparently indirect effect mediated by a distorted lateral pressure profile in the membrane suggests that autoinducers easily partition into the cytoplasmic membranes of these bacteria and may not require special transport systems for entry. Amenability to patch-clamp recording for the two new bacterial species opens broad opportunities for studies of solute permeability and electrogenic transport in their membranes.

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The Coupling of Tension and Crowding Sensing in the Bacterial Channel MscS
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Macromolecular excluded volume (crowding) is a critical parameter regulated by all cells, but how it is sensed remains unclear. Bacteria avoid dehydration in hypertonic media by accumulating ions and compatible osmolytes, which retain the necessary fraction of free water. Upon dilution of the medium, excess osmolytes are released through tension-activated channels acting as valves. We found that the mechanosensitive channel MscS, which exhibits slow inactivation under moderate tension, inactivates abruptly under the same tension in the presence of cytoplasmic crowders. To study the synergism between tension and crowding, we analyze the features that differentiate the inactivated (crystal-like) conformation from the modeled compact resting state, which include the splayed lipid-facing TM1-TM2 helices uncoupled from the gate and sharp kinks of the pore-lining TM3s at G113 stabilized by association of TM3b segments with the cytoplasmic beta domains. To mimic the effect of crowding, we performed MD simulations in which axial pressure is applied to the bottom of the cytoplasmic (cage) domain toward the membrane. We found that both models exhibit similar axial compliance and the cage reduces its volume in the cytoplasm, however in the inactivated state axial compaction produces a stronger expansion of the TM domain in the plane of the membrane. This concerted shape change appears to be the reason for the thermodynamic coupling of the bulk crowding effect with the energetic input from membrane tension, which both drive channel inactivation. We conclude that cage of MscS is the sensor which provides the feedback on increased crowding and disengages the gate to prevent c ‘over-draining’ of the cytoplasm. We discuss the mechanics of a bacterial cell surrounded by an elastic cell wall where this inhibitory feedback on osmolytes release might be necessary.